

Serological testing for *Bartonella henselae* infections in The Netherlands: clinical evaluation of immunofluorescence assay and ELISA

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ABSTRACT

Cat-scratch disease (CSD), caused by *Bartonella henselae* infection, can mimic malignancy and can manifest atypically. Reliable serological testing is therefore of great clinical importance. The diagnostic performance of immunofluorescence assay (IFA) and ELISA was evaluated in a group of Dutch patients with proven CSD (clinical diagnosis confirmed by PCR). Sera of 51 CSD patients and 56 controls (patients with similar symptoms, but who were *B. henselae* PCR-negative and had an alternative confirmed diagnosis) were tested for anti-*B. henselae* IgM and IgG by IFA and ELISA. A commercially available IFA test for IgM had a sensitivity of 6%. In-house assays for IgM showed specificities of 93% (IFA) and 91% (ELISA), but with low sensitivities (53% and 65%, respectively). With a specificity of 82% (IFA) and 91% (ELISA), in-house IgG testing showed a significantly higher sensitivity in IFA (67%) than in ELISA (28%, $p < 0.01$). Sensitivity was higher for genotype I (38–75%) than for genotype II (7–67%) infections, but this was only statistically significant for IgG ELISA ($p < 0.05$). In conclusion, detection of IgM against *B. henselae* by in-house ELISA and IFA was highly specific for the diagnosis of CSD. The high seroprevalence in healthy individuals limits the clinical value of IgG detection for diagnosing CSD. Given the low sensitivity of the serological assays, negative serology does not rule out CSD and warrants further investigation, including PCR. Adding locally isolated (e.g., genotype II) *B. henselae* strains to future tests might improve the sensitivity.

Keywords *Bartonella henselae*, cat-scratch disease, diagnosis, ELISA, immunofluorescence assay, PCR

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INTRODUCTION

Bartonella henselae is the causative agent of cat-scratch disease (CSD), which usually presents as a self-limiting lymphadenopathy. In a minority of cases, including immunocompromised hosts, *B. henselae* can cause atypical infections, such as osteomyelitis, endocarditis or peliosis hepatis. The prolonged painless lymphadenopathy may mimic malignancies and tuberculosis [1,2]. Quick and reliable confirmation of CSD can prevent unnecessary diagnostic procedures, or reveal

cases of CSD for which antibiotic treatment needs to be considered.

For over 30 years, diagnosis of CSD has relied on clinical criteria and skin tests [1], but the simplicity of serology means that this approach is now usually the first step in the confirmation of suspected CSD [3,4]. Indirect immunofluorescence assay (IFA) and ELISA are used for detection of anti-*B. henselae* antibodies in serum. Although IFA is the technique used most widely, IFA is more time-consuming than ELISA, and interpretation might be less objective [5]. Previous evaluations of serological tests reported a range of sensitivities and specificities, depending on the study population, definitions of CSD, and the materials and techniques used [3,6,7]. *B. henselae* is difficult to culture from patients, but PCR is

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highly specific and sensitive for detecting *B. henselae* DNA in pus or lymph node specimens. The sensitivity of PCR is dependent on the target genes and the particular patient group [3,8–12]. The main disadvantage of PCR is the need to obtain invasive samples of pus or other relevant tissue [10]. Although recent studies have suggested a more complex classification of *B. henselae*, two *B. henselae* 16S rRNA genotypes that are associated with cases of CSD have been identified previously [13–15]. Genotypes I (corresponding to serotype Houston-1) and II (serotype Marseille) can be distinguished by PCR [16]. After the discovery of genotype II, Drancourt *et al.* [13] suggested that the precise genotype involved might influence the accuracy of serological tests, although this has not been confirmed.

In the present study, the diagnostic performance of IFA and ELISA was evaluated in patients with a clinical presentation consistent with CSD, confirmed by PCR, and in a clinically relevant control group with negative PCR results. A commercially available IFA was compared with in-house assays, and the influence of the two different *B. henselae* genotypes on the sensitivity of these serological assays was examined.

MATERIALS AND METHODS

Patient population

Material from patients in The Netherlands with suspected *B. henselae* infection is sent to two national reference laboratories, the National Institute of Public Health and the Environment, Bilthoven (RIVM) and the Regional Public Health Laboratory, Tilburg (RPHL). Patients with material sent for both *B. henselae* PCR and serology, with sufficient serum stored for additional testing, were selected from the laboratory databases of both centres. The referring physicians were asked to complete an anonymised form that provided clinical and epidemiological data (age, gender, symptoms of disease, duration of illness, cat contact and final diagnosis). The patients were divided into the study group (CSD group) and the negative control group according to clinical data and PCR results (Fig. 1).

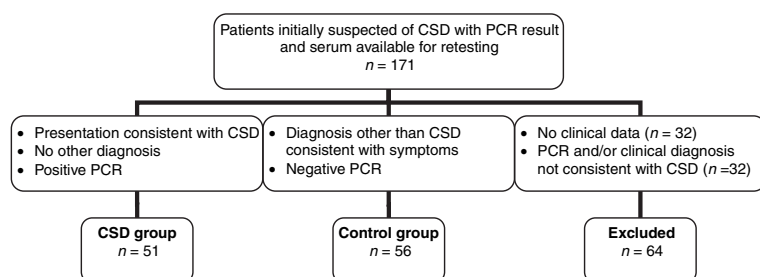


Table 1. Clinical diagnoses in the control group ($n = 56$)

Diagnosis	Number of patients (%)
Infection	20 (36%)
Mycobacterial (atypical)	8 (40%)
Mycobacterial (typical)	4 (20%)
Bacterial, other	7 (35%)
Viral	1 (5%)
Malignancy	21 (38%)
Hodgkin's lymphoma	6 (30%)
Non-Hodgkin's lymphoma	5 (25%)
Other	10 (50%)
Immunological disorder ^a	8 (14%)
Congenital cyst/fistula	5 (8.9%)
Other diagnosis	2 (3.6%)

^aReactive lymphadenitis in human immunodeficiency virus infection, rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, autoimmune lymphoproliferative syndrome and Devic's disease.

CSD group. The CSD group included patients with a clinical presentation of CSD, based on retrospective analysis of clinical data and a PCR test positive for *B. henselae*. Clinical presentation of CSD was defined as lymphadenitis or an atypical presentation of *B. henselae* infection in the absence of another diagnosis. The combination of matching clinical data and a positive PCR result was considered to be the reference standard for a proven infection with *B. henselae*.

Control group. The control group included patients whose material was sent for *B. henselae* testing, but who eventually had a different clinical diagnosis (Table 1) and a PCR test negative for *B. henselae*.

Exclusions. Exclusions included patients who did not meet the criteria for the above two groups, or for whom insufficient clinical data concerning their diagnoses were available.

Laboratory techniques

All serum samples were analysed for *B. henselae*-specific IgM and IgG antibodies by ELISA at RIVM, and by IFA at RPHL. If two or more serum samples from one patient were obtained ($n = 16$), the specimen collected nearest to the date of collection of PCR material was analysed. Sera were stored at -20°C .

IFA. In-house antigen slides for detection of IgM and IgG antibodies to *B. henselae* were prepared as described previously [3]. A bacterial suspension of $c. 10^8$ CFU/mL was made from *B. henselae* ATCC 49882 (*B. henselae* type Houston-1), grown on Columbia agar supplemented with sheep blood 5% v/v. The suspension was mixed with egg yolk emulsion

Fig. 1. Subdivision of patients initially suspected of having cat-scratch disease (CSD) on the basis of clinical analysis and PCR tests for *Bartonella henselae*, resulting in three groups: CSD group, control group and excluded patients.

50 µL/mL (Unipath, Basingstoke, UK), and spotted on Teflon-coated slides. The slides were air-dried for 30 min and fixed in acetone for 30 min. Sera and conjugates were diluted in phosphate-buffered saline (PBS; pH 7.2) containing bovine serum albumin 0.05% w/v and NaN₃ 0.1% v/v. For the detection of IgM antibodies, sera were pre-treated with IgG blocking solution (Incstar Corp., Stillwater, MN, USA).

The commercially available slides for detection of *B. henselae* IgM (Focus Technologies, Cypress, CA, USA) are marketed solely for in-vitro diagnostic use outside the USA. The commercially available slides were used according to the manufacturer's recommendations in parallel with the in-house prepared slides.

All IFA results were evaluated independently by two experienced scientists, who were unaware of the clinical and laboratory findings. Inter-observer differences never exceeded one dilution step. In cases of disagreement ($n = 11$), the opinion of an independent third scientist was considered conclusive. The most frequently reported cut-off values, i.e., positivity at titres of $\geq 1:64$ for IgG and $\geq 1:8$ for IgM, were used [17].

ELISA. Detection of anti-*B. henselae* IgM and IgG by an in-house ELISA was based on the methods described previously by Bergmans *et al.* [3] and Barka *et al.* [18], with several modifications to improve sensitivity. *B. henselae* strain ATCC 49882 was grown on Columbia agar plates containing sheep blood 5% v/v for 7–10 days at 35°C in an atmosphere containing CO₂ 5% v/v. Colonies were scraped from the plates, suspended in PBS, sonicated for 30 min and stored at –20°C. Wells of microtitre plates (Polysorb; Nunc, Roskilde, Denmark) were coated overnight at 4°C with 100 µL of an optimal dilution of the *B. henselae* antigen prepared in PBS. Parallel wells were coated with PBS alone to control for non-specific binding of serum components. After incubation, the plates were washed twice with PBS containing Tween-20 0.05% v/v and blocked with blocking reagent (Boehringer-Ingelheim, Ingelheim, Germany) according to the manufacturer's instructions. Serum dilutions (1:100) were added and then incubated for 1 h at 37°C. Plates were washed four times between each incubation step. For the IgM assay, sera were depleted of IgG in advance of testing with IgG-RF sorbent (Biotest, Shirley, UK), used according to the manufacturer's instructions, to prevent possible inter-isotype competition. Bound antibody was detected using horseradish-peroxidase-labelled goat anti-human IgM (Biorad, Hercules, CA, UK) or rabbit anti-human IgG (Dako, Glostrup, Denmark) for 1 h at 37°C. Tetramethylbenzidine was used as substrate, and colour development was stopped after 10 min with 2 M H₂SO₄. The plates were read at 450 nm (second filter 690 nm). A high positive, a low positive and a negative control serum were examined in each assay. In order to create a highly specific test, 126 age-matched controls for a group of PCR-positive CSD patients were used to calculate optimal cut-off values of the IgM and IgG ELISAs. Cut-off values were set at the average optical density (OD) for this group, plus three times the standard deviation (SD) for IgM, and plus two SD for IgG, to reach acceptable specificity levels of 97% and 98% for IgM and IgG, respectively. A serum sample was considered positive if the calculated ratio (OD sample:cut-off value) was ≥ 1 , which corresponded to cut-off values of ≥ 0.110 OD and 0.623 OD for IgM and IgG, respectively.

PCR. Pus aspirates and biopsy specimens from lymph nodes were used for PCR detection of *B. henselae* DNA, using primers based on the 16S rRNA gene [10]. This test was reported by Bergmans *et al.* [10] to have a sensitivity of 96% in patients with a positive skin test. If the *B. henselae* PCR was positive, a 16S rRNA genotype-specific PCR was performed to detect the type I and type II genotypes [16].

Data analysis

The data were analysed using SPSS v.11.0 for Windows (SPSS Inc., Chicago, IL, USA). Clinical and epidemiological data for the CSD and control groups were compared using the two-sample *t*-test or, when the normal distribution assumption was not met, the Mann-Whitney *U*-test for continuous data, and the chi-square test for nominal data. Sensitivity and specificity were calculated for detection of IgG and IgM in the IFA and ELISA tests. Receiver operating characteristic (ROC) curves were generated for the different tests, with the area under the curve (AUC) indicating the measure of accuracy.

As IFA results are expressed as titres, only a certain number of cut-off values was possible. Samples were tested only at a 1:8 dilution for IgM, and at 1:8, 1:16, 1:32 and 1:64 dilutions for IgG. Therefore, the ROC curve of the IFA test was angular, in contrast to the gradual line of the ELISA with its numerical results. Thus, comparing the AUCs for IFA and ELISA is not entirely appropriate for evaluation of the use of ELISA and IFA for testing IgM and IgG. In order to compare the two tests, the specificity of the ELISA was levelled with that of the IFA. Therefore, the cut-off for the ELISA tests was changed to attain the specificity of the IFA at cut-off titres of 1:8 (IgM) and 1:64 (IgG). Comparison of the sensitivity of the tests was then possible at equal specificity by use of McNemar's test for matched pairs for IgM and IgG. The sensitivity of serological testing was compared for different *B. henselae* genotypes. Group differences were considered to be statistically significant if their two-tailed *p* values were ≤ 0.05 .

RESULTS

Patient data

Between January 2000 and June 2001, 171 patients were selected, with a response rate of 86% to the clinical data enquiry. According to the clinical data and PCR results, the patients were divided into the CSD group and the negative control group (Fig. 1). Sixty-four patients were excluded because of insufficient or no clinical data ($n = 23$), an undefined diagnosis ($n = 13$), or a clinical presentation of CSD but a negative PCR result ($n = 28$).

CSD group. The median age of the CSD patients was 27.0 years ($n = 51$; range 1–83 years), which was not significantly different from that of the control group (median 31.2 years, range 1–81

years). No statistically significant difference in gender was found between the CSD group (71% male) and the control group (57% male). In the CSD group, most (96%) patients presented with typical CSD with lymphadenitis. In 45% of these cases, the lymphadenitis was axillary, with 31% being in the neck region. Two patients presented with atypical CSD (multifocal osteomyelitis and endocarditis) without lymphadenitis. Information concerning contact with cats was available for 33 CSD cases, with 88% reporting cat contact in the 3-month period before the start of symptoms, and 12% reporting no cat contact.

Control group. Patients in the control group ($n = 56$) were diagnosed with malignancies (38%), with infections other than CSD (36%), and with other clinical conditions (26%) (Table 1). In the control group, 87% of patients had lymphadenopathy, with the neck region being the most frequent (59%) localisation.

The period between collection of PCR and serum samples was <1 week for 47% of cases, and >10 weeks for 21% of cases, which did not differ significantly from the control group. To eliminate the possible influence of a large interval, the data were re-analysed after excluding the cases with an interval of >60 days ($n = 18$), but no change in the results was observed (data not shown).

Serology

The results obtained are summarised in Table 2.

In-house IFA. Specificities of 93% for IgM and 82% for IgG were reached with the in-house IFA, with sensitivities of 53% and 67%, respectively. Combining in-house IgM and IgG (defining positivity as a positive IgM and/or a positive IgG) increased the sensitivity to 86%, at the expense of a reduction in specificity to 77%.

Commercial IFA. Three of the 51 CSD patients tested positive with the commercial IFA IgM slides, corresponding to a sensitivity of 6%. All samples from the control group tested negative (specificity 100%).

In-house ELISA. The in-house ELISA showed a specificity of 91% for both IgM and IgG, and sensitivities of 65% and 28% for IgM and IgG, respectively. Combining the IgM and IgG results in the ELISA improved the sensitivity (77%), but reduced the specificity (82%).

Duration of disease

The date of the first clinical symptoms was known for 41 patients (44 sera) in the CSD group. The period between the first clinical symptoms and the time of blood sampling did not significantly influence the sensitivity or specificity of the assays, except for IFA IgM, which had a better sensitivity with blood samples taken within 6 weeks of the first symptoms. Despite the small numbers, Fig. 2 shows a pattern in serological positivity. In IFA, the IgM positivity decreased after 8 weeks, while IgG positivity peaked at 6–8 weeks. Such a pattern was not clear with the ELISAs, mainly because of the low sensitivity of the IgG ELISA.

Comparison of IFA and ELISA

IgM tests showed 86% concordance between the ELISA and IFA results. For IgG, concordance was 67%, mainly because of the difference in sensitivity. The ROC curves demonstrate that the IgG ELISA was poor at discriminating CSD patients from the control group, with an AUC of 0.67 (Fig. 3). The IgM and IgG IFAs performed better (AUC 0.73 and 0.79, respectively), and the IgM ELISA performed best (AUC 0.85). At the point where specificity is *c.* 92%, corresponding to the

Table 2. Results with *Bartonella henselae* immunofluorescence assay (IFA) and ELISA for cat-scratch disease (CSD) patients ($n = 51$) and the control group ($n = 56$), together with positive and negative predictive values

	IFA commercial assay		IFA in-house assay				ELISA in-house assay			
	IgM		IgM		IgG		IgM		IgG	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
CSD group n (%)	3 (6)	48 (94)	27 (53)	24 (47)	34 (67)	17 (33)	33 (65)	18 (35)	14 (28)	37 (73)
Control group n (%)	0 (0)	56 (100)	4 (7)	52 (93)	10 (18)	46 (82)	5 (9)	51 (91)	5 (9)	51 (91)
Predictive value (%)	100	54	87	68	77	73	87	74	74	58

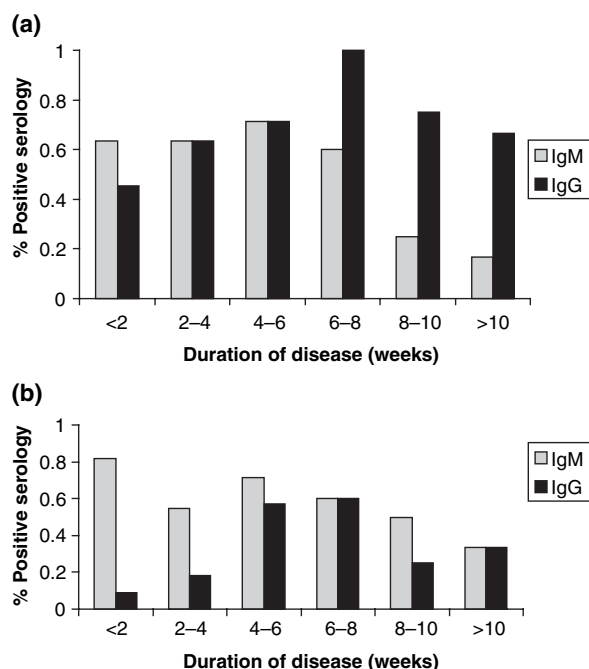


Fig. 2. Seropositivity with (a) in-house immunofluorescence assay (IFA) and (b) ELISA in relation to the duration of disease. The percentage of positive sera ($n = 44$) by IFA and ELISA is shown for *Bartonella henselae* IgG and IgM in relation to duration of the disease in weeks. The numbers of sera are 11, 11, seven, five, four and six, respectively, for each pair of bars.

cut-offs used in this study, the curves of these last three tests were nearly superimposed.

In order to compare the sensitivities of the IFA and ELISA, the ELISA cut-off was changed to meet the same specificity as the IFA test. The specificity of the IgM IFA (cut-off 1:8) was 92.9%, which was also met in the ELISA after shifting the ELISA cut-off value to 4.89 SD. At this point, the sensitivity of the IgM ELISA (49%) did not differ significantly from that of the IFA (53%). For the IgG tests, equal specificity (82%) was reached at a cut-off of 1:64 (IFA) and 1.78 SD (ELISA). With this cut-off, IgG sensitivity was significantly higher for the IgG IFA (82.1%) than for the IgG ELISA (29.4%) ($p = 0.003$). The ROC curve illustrates that the commercially available IgM IFA did not discriminate well, with an AUC of 0.53.

B. henselae genotyping

All lymph node or pus specimens from the patients in the CSD group ($n = 51$) were retested with a PCR that amplified a genotype-specific 185-bp 16S rRNA fragment. Four samples were

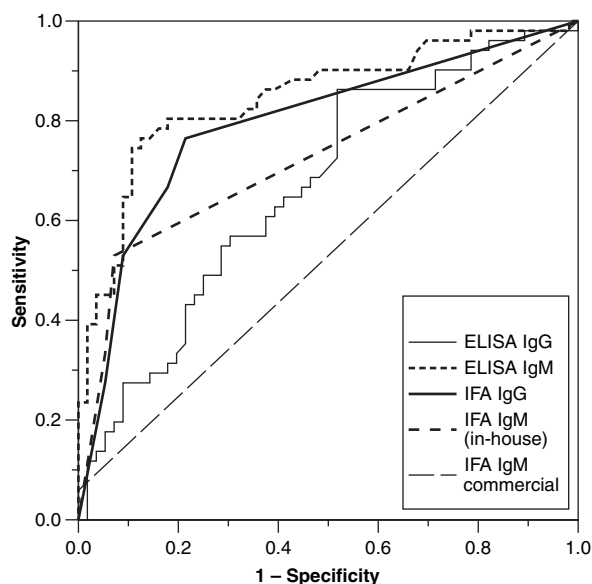


Fig. 3. Receiver operating characteristic curves for serological testing of *Bartonella henselae* infection in patients suspected clinically of having cat-scratch disease. Areas under the curve (95% CI) are 0.85 (0.77–0.92) and 0.67 (0.57–0.77) for IgM and IgGs ELISAs, and 0.73 (0.63–0.83) and 0.79 (0.71–0.89) for in-house IgM and IgG immunofluorescence assays (IFAs), respectively. The area under the curve for the commercially available IgM IFA is 0.53 (0.42–0.64).

not included in this analysis, either because the DNA concentration was too low ($n = 3$), or because genotypes I and II were both identified ($n = 1$; possible co-infection). Genotype I was identified in 32 (63%) lymph node samples and genotype II in 15 (29%) samples. Table 3 shows that the sensitivity of the serological tests was higher for type I than for type II *B. henselae*, but this was only statistically significant for the IgG ELISA ($p = 0.028$).

DISCUSSION

The present study evaluated the diagnostic performance of *B. henselae*-specific IgM and IgG IFAs and ELISAs, using a group of patients with proven CSD (clinical diagnosis confirmed by PCR) and a control group of patients with similar symptoms, but with other confirmed diagnoses and PCR results negative for *B. henselae*. In-house IgM assays showed a low sensitivity (IFA 53%, ELISA 65%) with a high specificity (IFA 93%, ELISA 91%). IgG assays were more sensitive in IFA (67%) than in ELISA (28%), with specificities of 82% and 91%, respectively. Two cases without

Table 3. Sensitivity of serological tests in relation to *Bartonella henselae* genotype

Serological test	Genotype I-positive sera (<i>n</i> = 32)	Genotype II-positive sera (<i>n</i> = 15)	P
IFA			
IgM	66%	40%	0.10
IgG	69%	67%	0.89
ELISA			
IgM	75%	53%	0.14
IgG	38%	7%	0.028

^aChi-square test.

IFA, immunofluorescence assay.

lymphadenopathy were included in the CSD group. One patient presented with multifocal osteomyelitis, as described by de Kort *et al.* [19], while the other had *B. henselae* endocarditis, which usually presents as a fever of unknown origin without lymphadenopathy in immunocompromised patients or in patients with a pre-existing cardiac defect. Although it is debatable whether *B. henselae* endocarditis should be considered as atypical CSD, it is believed that the inclusion of this patient did not flaw the overall analysis.

Compared with other reports, relatively low specificities were achieved, caused by a high seroprevalence in the control group. This could be explained in several ways. The controls, all of whom were diagnosed with diseases other than CSD, could still have had a concurrent *B. henselae* infection. Rolain *et al.* [20] described mycobacteriosis in 4.2% and neoplasm in 1.2% of proven CSD patients (*n* = 245). Negative PCR results may have been caused by a delay in sample taking, reduced DNA availability, or the limited sensitivity of the assay (78–100%) [3,10,12].

The IgG results positive for *Bartonella* in the control group (seroprevalence 9–18%) may reflect past exposure to *B. henselae*, as IgG remains positive long after infection [3,5]. Although serological cross-reactivity with *B. henselae* has only been reported for *Bartonella quintana* and *Coxiella burnetii* [21], cross-reactivity with other microorganisms is not unlikely [4,8]. In clinical practice, tests for diagnosing CSD need a high specificity, as potentially lethal diseases, e.g., malignancies and tuberculosis, can be missed in cases in which CSD is diagnosed incorrectly [7]. Given the high seroprevalence and low sensitivity of testing for anti-*B. henselae* IgG (especially by ELISA), IgG testing appears to be of no additional value in diagnosing acute CSD.

Despite the low numbers, it was confirmed that IgM antibodies are present predominantly in the early phase of disease, while IgG antibody titres increase subsequently [3,5,22,23]. IgM sensitivity was optimal in the first 6 weeks after onset of symptoms. Earlier reports have described variable patterns of antibody kinetics, but often with an unknown duration of the disease at the time of sample taking [3,5,22,23]. Metzker-Cotter *et al.* [5] analysed ELISA antibody kinetics in 98 patients with CSD symptoms for 1–52 weeks, with a mean serological follow-up of 35 weeks (range 2–211 weeks), and revealed that IgM seropositivity disappeared within 3 months in 96% of the patients, while IgG titres may remain positive for >2 years after the onset of disease. The present study suggests that serological confirmation of CSD is best performed 6–8 weeks after the onset of disease. In patients with suspected CSD and negative serology, a *B. henselae* PCR with relevant material should be performed because of the low sensitivity of serological testing [3,11].

It is difficult to compare the present results with earlier findings, because of the different study populations and definitions of CSD. Various materials and techniques have been evaluated, e.g., different commercially available vs. in-house IFAs or ELISAs, detection of IgM or IgG, and the use of different cut-off values [3–7,10]. However, Bergmans *et al.* [3] reported sensitivities for IgM of 50% (IFA) and 71% (ELISA), and of 81% (ELISA), in PCR-positive patients, but as in the present study, a low sensitivity for IgG (9.5% ELISA, 41% IFA). Giladi *et al.* [6] reported that an in-house ELISA, using the same cut-off values as the present study, had sensitivities for IgM and IgG of 48% and 77%, and specificities of 100% and 94%, respectively.

Comparison of serological tests from different laboratories is also complicated by the lack of standardisation. Therefore, the in-house IgM IFA was compared with a commercially available IgM IFA (Focus Technologies). The product information brochure reported a specificity of 100% (tested in 75 USA blood donors) and a sensitivity of 41.5% in 94 CSD patients with lymphadenopathy and cat exposure. However, in the present study, this test had a sensitivity of only 6%. In an earlier report, Maurin *et al.* [24] compared the Focus Technologies IgM and IgG IFAs with an in-house IFA. In a group of 68 French CSD patients (with cat contact and positive PCR results), a

sensitivity of 1.5% (1/68) was found for the commercial IgM test, with a specificity of 100% in healthy blood donors ($n = 40$). The in-house IgM IFA performed similarly, with a sensitivity of 3% (2/68) at a cut-off titre of $\leq 1:20$. The IgG IFA sensitivity was higher for the commercial test (91%) than for the in-house assay (53%, $p < 0.001$), with a specificity of 100% in healthy blood donors and of 70% in patients with tuberculosis ($n = 10$).

Reports concerning other commercially available tests illustrate the range in performance of serological tests for *B. henselae*. Zbinden *et al.* [25] reported that two commercial IFAs with agar-derived *B. henselae* showed IgG positivity with 45–52% of healthy controls ($n = 58$), while two other IFAs with cell-associated *B. henselae* were positive in only 3.4–5.2% of cases. Sander *et al.* [7] evaluated two IFAs, one based on larynx carcinoma cells infected with *B. henselae* (type Houston-1) and one based on Vero cells infected with *B. henselae* and *B. quintana*. In a group of 42 CSD patients (20 PCR-positive) and 100–270 healthy controls, IgM tests were 88–95% sensitive and 64–86% specific, while IgG tests were 93–100% sensitive and 70–73% specific. An antibody seroprevalence to *B. henselae* of 30% was measured in healthy German individuals, irrespective of cat ownership.

Whether the poor performance of the commercial IgM test in the present study, as well as in the French study, was caused by a failure in slide preparation or by the characteristics of the study population remains unclear [24]. Another explanation might be the influence of different genotypes on the sensitivity, as shown in the present study. Although the number of patients was too low to achieve statistical significance (except for the IgG ELISA), infection with a genotype I strain seemed to be detected more easily by current serological techniques than were infections with type II strains. Possibly an infection with a genotype I strain triggers a higher and longer-lasting antibody response than that obtained with genotype II strains, and is therefore detected more easily. However, a more plausible explanation is the use of the homologous ATCC 49882 (genotype I) *B. henselae* strain in the IFA and ELISA, which might give a lower or negative serological reactions in patients infected by genotype II [13]. Since c. 25% of Dutch CSD cases are not infected with *B. henselae* type I, the use of locally isolated 16S type I and type II *B. henselae* strains in serological assays may improve performance [16].

In conclusion, improving serological tests for diagnosing CSD remains a challenge, as no ideal serological test has yet been described. In this population, the detection of *B. henselae* IgM antibodies by IFA or ELISA in patients suspected of having CSD is highly confirmative for the diagnosis, with a positive predictive value of 87%. IgG tests appear to be of limited use, as sensitivity and specificity will be low in populations with a high seroprevalence. Given the low sensitivity of the serological assays, PCR analysis for *B. henselae* should be considered for patients with suspected CSD and negative serology. As the sensitivity of serological testing seems to depend on the *B. henselae* genotype causing the infection, the inclusion of several locally isolated strains might improve the performance of the tests.

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